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EXPERIMENTAL STUDY

Effects of Sini San used alone and in combination with fluoxetine on central and peripheral 5-HT levels in a rat model of depression

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Abstract

OBJECTIVE: To investigate the effects of Sini San and fluoxetine on the levels of central and peripheral 5-HT in a rat model of depression, and provide new insight into the treatment of depression with integrated Chinese-Western Medicine.

METHODS: A rat model of depression was established by chronic mild stress (CMS). Model rats received either Sini San, fluoxetine, a combination of the two drugs, or no drug treatment. Healthy naive rats were used as controls. Open field and sucrose preference tests were used to assess depression-like behavior. ELISA and immunohistochemistry were used to determine central and peripheral levels of 5-HT.

RESULTS: In the group with no drug treatment, central 5-HT expression decreased while peripheral 5-HT concentrations increased as CMS continued. Four weeks after CMS, Sini San alone was less effective in reducing depression-like behavior than fluoxetine alone or in combination with Sini San, but combined use was more effective than fluoxetine alone. Eight weeks after CMS, Sini San alone or in combination with fluoxetine was more effective in reducing depression-like behavior than fluoxetine alone. Furthermore Sini San and fluoxetine used alone or in combination notably increased central 5-HT expression and decreased peripheral 5-HT levels in the rat model.

CONCLUSION: The results of the present study indicate that there is a synergistic action between the two medicines in the treatment of depression. Sini San exhibited a relatively long lag before its effects were observed; however, by eight weeks the Traditional Chinese Medicine appeared at least as effective as fluoxetine. We suggest that Sini San can replace fluoxetine in the later stages of depression treatment to minimize side effects observed with long-term fluoxetine administration.

Key words: Fluoxetine; Stress; Depression; Serotonin; Sini San

INTRODUCTION

Depression is a mood disorder characterized by persistent low mood, decreased motivation, and apathy. The suicide rate in depressed patients is 20 times higher than that in the normal population.[1] Modern medical investigation has demonstrated that the onset of depres-

sion is related to a lack of serotonin (5-HT),[2] a neurotransmitter involved in the regulation of emotion, memory, appetite and sexual function.

Chronic mild stress (CMS) is a widely accepted method of establishing a rat model of depression. In this paradigm, animals are exposed to a series of unpredictable mild stressors which reflect the varied, low-intensity environmental triggers that can lead to the development of depression. As exposure to CMS continues, the animals' consumption of food and water decreases, reflecting a core symptom of depression, anhedonia,[3,4] and mimicking the onset and development of depression in man.

Fluoxetine is currently a first-line treatment for depression in Western Medicine; however, serious side effects may occur after long-term use. Therefore, Traditional Chinese Medicine or integrated traditional Chinese and Western Medicine are widely adopted in the clinical treatment of depression in China. Sini San is commonly prescribed for the treatment of depression in Traditional Chinese Medicine. In the present study, we observed the effects of Sini San and fluoxetine, administered alone or together, on the behavior and central and peripheral 5-HT levels in rats exposed to CMS, to provide new insight into the application of integrated traditional Chinese and Western Medicine for the treatment of depression.

MATERIALS AND METHODS

Experimental animals and grouping

A total of 200 male, specifically pathogen-free Sprague Dawley rats, aged 6 weeks and weighing (200 ± 10) g, were provided by Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. (experimental animal production license: SCXK Beijing 2006-0009). The rats were fed and housed in the animal room of the Scientific Research Center of Beijing University of Chinese Medicine, at an ambient temperature of 20℃-25℃ and under a 12 h light/dark cycle. Rats were habituated to the housing room for two weeks, then randomly divided into five groups by random number table method (n=40 per group). Four groups were exposed to CMS: a stress-only group (exposed to CMS with no subsequent drug treatment); a Sini San group (exposed to CMS then received Sini San); the fluoxetine group (CMS then fluoxetine); and a combined Sini San and fluoxetine group (CMS then combined administration of Sini San and fluoxetine, hereinafter referred to as the combination group). Naive rats were used as controls. All rats in the test groups were housed in isolation. Control rats were housed in groups of three, except during the sucrose preference test when they were also housed singly.

Reagents

Rat 5-HT enzyme-linked immunosorbent assay (ELISA) kits were purchased from American R&D Co. (Minneapolis, MN, USA); the primary antibody (mouse anti-rat 5-HT monoclonal IgG) was purchased by Santa Cruz Biotechnology, Inc (Dallas, TX, USA); the secondary antibody (biotin-labeled goat anti-mouse IgG) and the immunohistochemistry detection kits were purchased by Gene Technology Co., Ltd. (Shanghai, China).

Establishment of a rat model of depression by exposure to CMS

Rats were exposed daily to a random selection of three stressors from the following: no food or water for 24 h; cage tilted at 30° overnight; wet housing overnight; 5 min swim in water at 4℃; heating of home cage to 45℃ for 5 min; restraint for 2 h; tail clamping, in which a hemostatic clamp was placed on the tail near the base for 1 min, with enough pressure to cause the rat to vocalize; and 36 sessions of electrical stimulation that was administered as inescapable foot shock in a chamber at a 1.5 mA intensity consisting of 30 shocks in 1 min, with an interval of 30 s between sessions. CMS was carried out for four weeks in the first experiment, and eight weeks in the second experiment. The CMS started and ended at the same time for the all groups.

Sini San and fluoxetine administration

Sini San, composed of Chaihu (*Radix Bupleuri Chinensis*), Zhishi (*Fructus Aurantii Immaturus*), Chishao (*Radix Paeoniae Rubra*) and Gancao (*Radix Glycyrrhizae*), was purchased from China Beijing Tongrentang Group Co., Ltd. (Beijing, China), and identified according to the Pharmacopoeia of the People's Republic of China (2010 edition) by the China-Japan Friendship Hospital pharmacy in Beijing. Each ingredient (200 g) was broken into soybean-like particles, soaked for 24 h in 95% alcohol, and refluxed and extracted three times per hour in 95% alcohol. The extracts were then concentrated and resuspended in 1000 mL 0.5% sodium carboxymethyl cellulose to make a Sini San suspension containing each ingredient at a concentration of 0.24 g/mL. The human to rat dose conversion coefficient is 0.16,[5] assuming body weights of 60 kg for an adult human and 0.2 kg for an adult rat. Human doses are 6 g/day for each ingredient, so an equivalent dose in the rat is 0.12 g for each ingredient. Fluoxetine was purchased as 20 mg capsules from Lilai Suzhou Pharmaceutical Co., Ltd. (Jiangsu, China) and a 10 mg/mL solution in normal saline was prepared. Immediately before the daily exposure to CMS, rats in the Sini San group were fed with Sini San suspension (2 mL/kg), those in the fluoxetine group were given fluoxetine (2.5 mL/kg i.p.), and those in the combination group received both Sini San (2 mL/kg p.o.) and fluoxetine (2.5 mL/kg i.p.).

Sucrose preference test

The sucrose preference test was carried out every two weeks, starting two weeks prior to CMS exposure. Animals were deprived of food and water for 12 h before the test. Two 100 mL bottles of sucrose solution (2%)

were placed in each cage for 12 h; 12 h later the bottles were changed to one 100 mL bottle of sucrose (2%) and one 100 mL bottle of water. The bottles were then weighed to determine the amount of sucrose, water and total liquid consumed,[6] and the preference percentage of sucrose was calculated according to the formula: preference percentage=(sucrose consumption/total liquid consumption)×100%.

Ethological observation

A square open field was used, measuring 100×100 cm with black walls 35 cm high and a 4×4 cm grid drawn on the floor. A digital camera was placed 2 m above the open field to capture the whole field. During the test, the rats were placed in the center of the field and recorded using a small animal behavior recording and analysis system (Panlab Smart version 1.0) for 3 min. Locomotor activity was determined from the frequency of gridline crosses and the total distance moved. In order to prevent animals habituating to the open field, the test was carried out every four weeks from the start of CMS exposure.

Blood and tissue sampling

A 2 mL blood sample was collected from animals in each group using retro-orbital bleeding, before CMS and 2, 4 and 6 weeks after the start of CMS. Twenty rats were randomly selected from each group to obtain brain tissue samples after 4 weeks of CMS. For the remaining rats, brain tissue was obtained after 8 weeks of CMS; rats were anaesthetized with 10% chloral hydrate, 5 mL of abdominal blood was taken, and the brain and small intestine were immediately removed on ice. Tissues were fixed in 4% paraformaldehyde for 48 h, and then embedded in paraffin. Hippocampal CA2 was examined at the following coordinates relative to bregma: 3.8 mm posterior, 5.20 mm lateral.

Determination of 5-HT serum levels

The ELISA kit (American R&D Co., Minneapolis, MN, USA) was brought to room temperature, 10 μL of serum from each animal was added to the wells. A standard curve was prepared by adding 10 μL of protein standards of known concentrations to separate wells. Mouse anti-rat 5-HT IgG (40 μL, Santa Cruz Biotechnology Inc, Dallas, TX, USA) at 1∶200 dilution was added to each well and biotinylated anti-rat IgG (40 μL) were then added to each well. The plate was sealed and shaken gently for 30 s before incubation at room temperature for 45 min. The liquid was removed, and the plate was washed by adding 350 μL washing liquid to each well and removing it, a total of five times. Substrate solution (100 μL) was added to each well for color development and the plate was gently mixed for 10 seconds before incubation at room temperature for 20 min. The reaction was terminated using 100 μL stop solution and shaking the plate gently for 30 s. The optical density (OD) of each well was read at 450 nm within 30 min of terminating the reaction. Serum sample concentrations were determined by comparing the OD of each sample against the standard curve.

Determination of 5-HT expression in the brain and small intestine

Expression of 5-HT in the brain and small intestine was detected by immunohistochemistry. The paraffin-embedded sections were preheated and dewaxed, then dehydrated in a graded series of alcohols, and microwaved for 6 min, After cooling to room temperature, the sections were placed into 0.3% H_2O_2 for 15 min, rinsed with phosphate-buffered saline (PBS) for 5 min three times, and incubated overnight in mouse anti-rat 5-HT IgG at 4℃. After rinsing again with PBS, the sections were then incubated with horseradish peroxidase-labeled secondary antibody for 1 h at room temperature. DAB was used to visualize staining, and the sections were counterstained with hematoxylin, dehydrated, cleared and sealed. For the negative control, sections were incubated without primary antibody with all remaining steps carried out as described. Image-Pro Plus version 6.0 (Media Cybernetics, Inc., Bethesda, MD, USA) image analysis software was used to analyze the data. Three brain sections and three small intestine sections were selected for each rat, and four non-overlapping views in the hippocampal CA2 region were selected for each section under a light microscope at 200× magnification. Mean OD for each animal was the value included in the analysis.

Statistics

All data are expressed as mean ± standard derviation (*SD*). SPSS 17.0 software (SPSS v.17.0 for Windows; SPSS Inc., Chicago, IL, USA) was used to analyze the data. Groups were compared using a two-way analysis of variance (ANOVA). The least significant difference (LSD) post-hoc test was used for data with homogeneity of variance, and Tamhane's T2 test was used for those with heterogeneity of variance. $P<0.05$ was considered statistically significant.

RESULTS

Sucrose preference

Before CMS exposure there were no differences in sucrose preference among the five groups ($P>0.05$). After 2 weeks of CMS, sucrose preference by rats that had received Sini San, fluoxetine, or both combined, was lower than that in the naive control group ($P=0.040$). After 4 weeks of CMS, sucrose preference in the Sini San group and the stress-only group was lower than that in the naive control group ($P=0.035$); sucrose preference in the fluoxetine and combination groups was not significantly different from the naive control group ($P>0.05$), but higher than that in the stress-only group ($P=0.022$); rats that had received Sini San consumed less

sucrose than those in the fluoxetine group (P=0.025). After 6 weeks of CMS, the consumption in the Sini San group remained lower than that in the naive control group and the fluoxetine group (P=0.012), but was higher than that in the stress-only group (P=0.023); the fluoxetine and combination groups showed no significant difference in sucrose preference compared with the normal control group (P>0.05), but consumed more than the stress-only group (P=0.048). After 8 weeks of CMS, the Sini San, fluoxetine, and combination groups showed no significant differences compared with the naive control group (P>0.05), and had a higher level of consumption than the stress-only group (P=0.003). The changes observed within each group as CMS continued were as follows: in the stress-only group, sucrose preference decreased gradually throughout the experiment (P=0.025); in the Sini San group, consumption reduced until 4 weeks (P=0.046) but rose again between 4 and 8 weeks of CMS (P=0.019); consumption in the fluoxetine group reduced after 2 weeks of CMS (P=0.020) rose again after 4 weeks (P=0.031) and reached a plateau at 6-8 weeks of CMS (P>0.05); the pattern of change in sucrose preference in the combination group followed that in the fluoxetine group, reducing after 2 weeks of CMS (P=0.039), increasing after 4 weeks (P=0.022) and stabilizing from 6 weeks until the end of the experiment (P>0.05) (Figure 1).

Locomotor activity

Before the stress, no significant differences were found among the five groups of rats in the frequency of gridline crosses or the total distance moved in the open field (P>0.05). After 4 weeks of CMS exposure, the gridline-crossing frequency and the total distance moved in the stress-only group, Sini San group, fluoxetine group and combination group were significantly lower than those of the naive control group (P=0.034); the gridline-crossing frequency and the total distance moved by rats in the Sini San group, fluoxetine group and combination group were significantly higher than those in the stress-only group (P=0.021), but both parameters were lower in the Sini San group than in the fluoxetine group (P=0.013), and no significant difference was found between the combination and fluoxetine groups (P>0.05). After 8 weeks of CMS, the gridline-crossing frequency and total distance moved in the stress-only group, Sini San group, fluoxetine group and combination group were significantly lower than those in the naive control group (P=0.027); the gridline-crossing frequency and total distance moved in the Sini San group, fluoxetine group and the combination group were significantly higher than those in the stress-only group (P=0.032), with no significant differences among the three drug-treated groups (P>0.05). Progression over time in the two locomotor activity parameters between 4 and 8 weeks of CMS within each group was as follows: gridline-crossing frequency and total distance moved in stress-only rats decreased at both time points (P=0.030); the Sini San group showed a decrease in both parameters at 4 weeks after CMS (P=0.022) and a rise again at 8 weeks (P=0.029); the fluoxetine and combination groups showed a decrease in locomotor activity 4 weeks after the stress (P=0.042) which remained stable until eight weeks (P>0.05) (Tables 1 and 2).

Hippocampal expression of 5-HT

After 4 weeks of CMS, 5-HT expression in the hippocampal CA2 region was determined in 20 rats of each group using immunohistochemistry. Levels of 5-HT in the four experimental groups were lower than in the

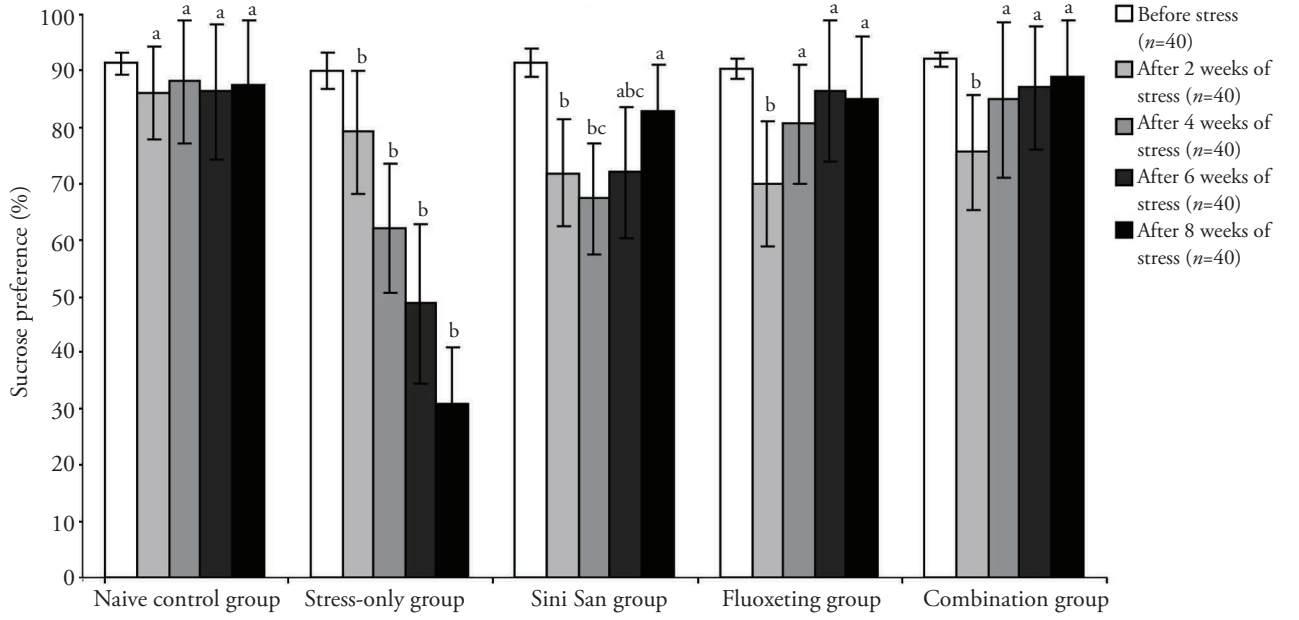

Figure 1 Sucrose preference (%) in rats undergoing chronic mild stress

Naive rats were used as controls; the stress-only group were exposed to chronic mild stress (CMS) with no subsequent drug treatment; the Sini San group were exposed to CMS then received Sini San; the fluoxetine group were exposed to CMS then fluoxetine; the combination group were exposed to CMS then combined administration of Sini San and fluoxetine.

naive control group (P=0.024); those in the Sini San group were not significantly different from those in the stress-only group (P>0.05), while the remaining groups had higher 5-HT expression levels than the stress-only group (P=0.036); Sini San-treated rats showed lower 5-HT levels than the fluoxetine group (P=0.011), whereas the combination group was not different from the fluoxetine group (P>0.05). After 8 weeks of stress, 5-HT expression in CA2 was determined in the remaining 20 rats in each group. Expression levels in the four experimental groups were lower than those in the naive control group (P=0.034); the Sini San, fluoxetine and combination groups showed significantly greater 5-HT expression than the stress-only group (P=0.008); 5-HT expression in the Sini San and combined groups was significantly higher than that in the fluoxetine group (P=0.017). Changes observed in 5-HT expression within each group between 4 and 8 weeks post-CMS were as follows: a decrease in the stress-only group (P=0.036), an increase in the Sini San group (P=0.025), and no change in the fluoxetine, combination and control groups (P>0.05) (Table 2 and Figure 2).

5-HTexpression in small intestine

After 4 weeks of stress, the expression of 5-HT in the small intestine was measured in 20 rats of each group using immunohistochemistry. The four experimental groups showed greater expression of 5-HT than the control group (P=0.001); expression levels in the Sini San group were not significantly different from those in the stress-only group (P>0.05); the levels of 5-HT in the fluoxetine and combination groups were significantly lower than those in the stress-only group (P=0.029); in the Sini San group, the expression of 5-HT was significantly higher than that in the fluoxetine group (P=0.017); and 5-HT expression in the fluoxetine group was not significantly different from that in the combination group (P>0.05). After 8 weeks of stress, 5-HT expression in the small intestine was measured in the remaining 20 rats of each group. The levels of 5-HT in the four experimental groups were higher than those in the naive control group (P=0.027); the Sini San, fluoxetine and combination groups had significantly lower 5-HT expression than the stress-only group (P=0.036); the levels of 5-HT in the Sini San group and the combination group were lower than those in the fluoxetine group (P=0.041). The changes in 5-HT expression in the small intestine within each group from 4 to 8 weeks after the stress were as follows: an increase in the stress-only group (P=0.022), a decrease in the Sini San and fluoxetine groups (P=0.014); and no significant change in the combination or naive control groups (P>0.05) (Table 3 and Figures 3).

Serum 5-HT concentrations

Before CMS, no significant differences were found in serum 5-HT content among the five groups of rats (P>

Table 1 Gridline-crossing frequency (total number of crosses in 3 min

Group	Before stress (n=40)	4 weeks after stress (n=40)	8 weeks after stress (n=20)
Naive control group	71.8±6.7	73.5±7.3[a]	79.8±6.3[a]
Stress-only group	76.1±6.3	17.2±3.0[b]	12.8±2.4[b]
Sini San group	77.4±2.0	41.8±6.8[abc]	59.8±9.5[ab]
Fluoxetine group	79.6±9.1	59.1±12.5[ab]	58.2±11.2[ab]
Combination group	73.6±3.0	58.2±10.6[ab]	60.7±13.9[ab]

Notes: naive rats were used as controls; the stress-only group were exposed to chronic mild stress (CMS) with no subsequent drug treatment; the Sini San group were exposed to CMS then received Sini San; the fluoxetine group were exposed to CMS then fluoxetine; the combination group were exposed to CMS then combined administration of Sini San and fluoxetine. [a]P<0.05, compared with the stress-only group; [b]P<0.05, compared with the normal control group; [c]P<0.05, compared with the fluoxetine group.

Table 2 Mean optical density of 5-HT in hippocampal CA2 in rats undergoing chronic mild stress (n=20)

Group	After 4 weeks of stress	After 8 weeks of stress
Naive control group	431±26[a]	497±50[a]
Stress-only group	308±36[b]	123±32[b]
Sini San group	321±24[bc]	402±26[abc]
Fluoxetine group	391±50[ab]	372±35[ab]
Combination group	398±46[ab]	410±70[abc]

Notes: naive rats were used as controls; the stress-only group were exposed to chronic mild stress (CMS) with no subsequent drug treatment; the Sini San group were exposed to CMS then received Sini San; the fluoxetine group were exposed to CMS then fluoxetine; the combination group were exposed to CMS then combined administration of Sini San and fluoxetine. [a]P<0.05, compared with the stress-only group; [b]P<0.05, compared with the control group; [c]P<0.05, compared with fluoxetine group.

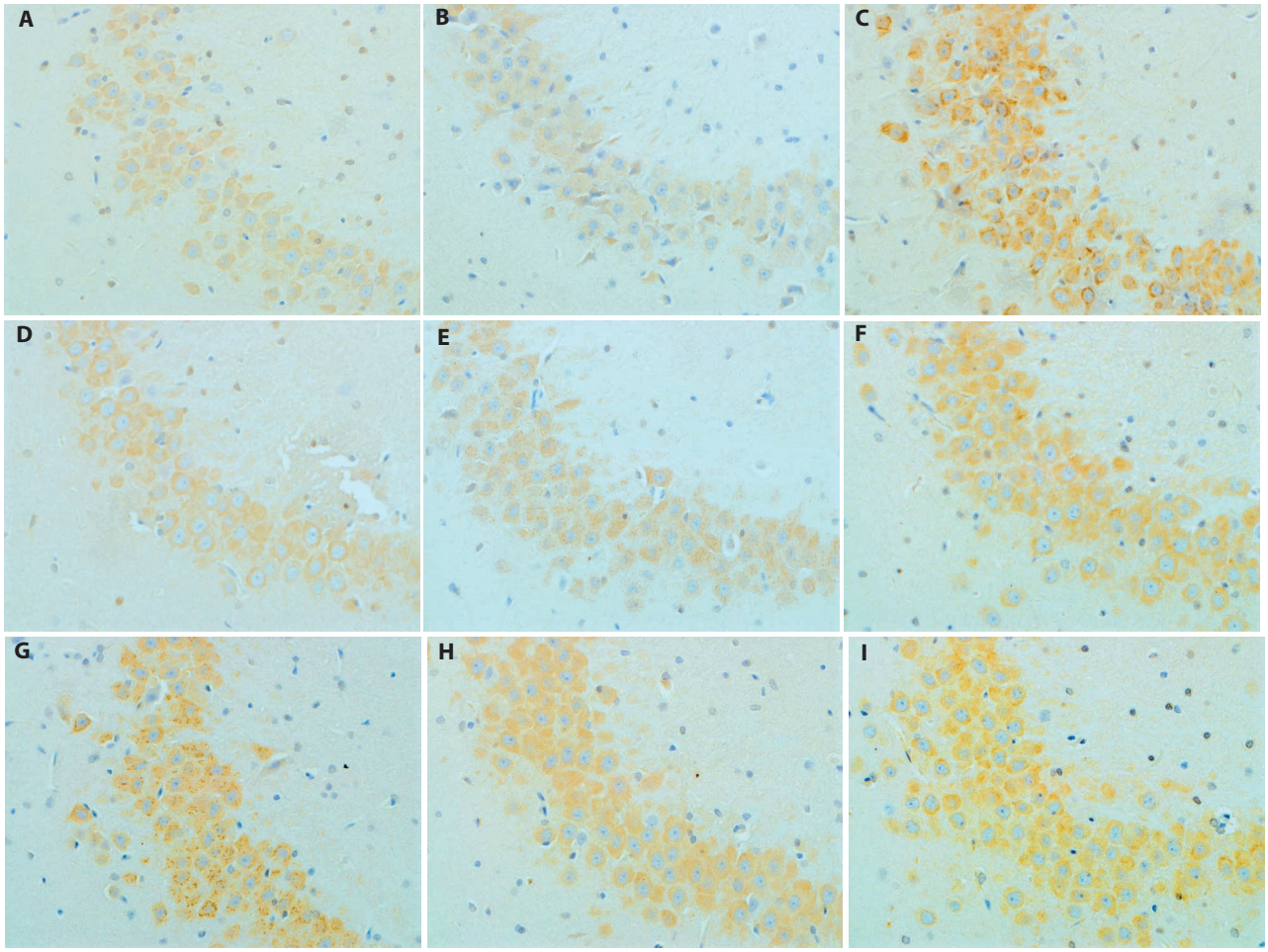

Figure 2 Expression of 5-HT in rat hippocampal CA2 (Immunohistochemical staining, × 200)
A: stress-only group after 4 weeks of stress; B: stress-only group after 8 weeks of stress; C: naive control group; D: Sini San group after 4 weeks of stress; E: fluoxetine group after 4 weeks of stress; F: combination group after 4 weeks of stress; G: Sini San group after 8 weeks of stress; H: fluoxetine group after 8 weeks of stress; I: combination group after 8 weeks of stress.

Table 3 Mean optical density values of 5-HT in rat small intestine

Group	After 4 weeks of stress (n=20)	After 8 weeks of stress (n=20)
Naive control group	68±20[a]	87±37[a]
Stress-only group	234±42[b]	495±152[b]
Sini San group	251±60[bc]	109±132[abc]
Fluoxetine group	101±61[ab]	126±95[ab]
Combination group	93±39[ab]	104±61[abc]

Notes: naive rats were used as controls; the stress-only group were exposed to chronic mild stress (CMS) with no subsequent drug treatment; the Sini San group were exposed to CMS then received Sini San; the fluoxetine group were exposed to CMS then fluoxetine; the combination group were exposed to CMS then combined administration of Sini San and fluoxetine. [a]P<0.05, compared with the stress-only group; [b]P<0.05, compared with the control group; [c]P<0.05, compared with the fluoxetine group.

0.05). After 2 weeks of stress, serum concentrations of 5-HT in all four experimental groups were higher than in the naive control group (P=0.002), with a lower concentration observed in the combination group than in the stress-only and fluoxetine groups (P=0.036). After 4 weeks of stress, serum levels of 5-HT in all experimental groups were higher than in the naive control group (P=0.041); rats treated with fluoxetine or both drugs in combination had lower serum concentrations of 5-HT than those in the stress-only group (P=0.020), and 5-HT serum concentrations in rats of the Sini San group were not significantly different from those of the stress-only group (P>0.05) but higher than those of the fluoxetine group (P=0.031). After 6 weeks of stress, serum 5-HT levels were still higher in the Sini San group than in the control and fluoxetine groups (P=0.014), with no significant difference from the stress-only group (P>0.05); and the fluoxetine and combination groups had higher 5-HT levels than the control group (P>0.05). After 8 weeks of stress, serum 5-HT levels in the Sini San and combination groups were significantly lower than those in the stress-only

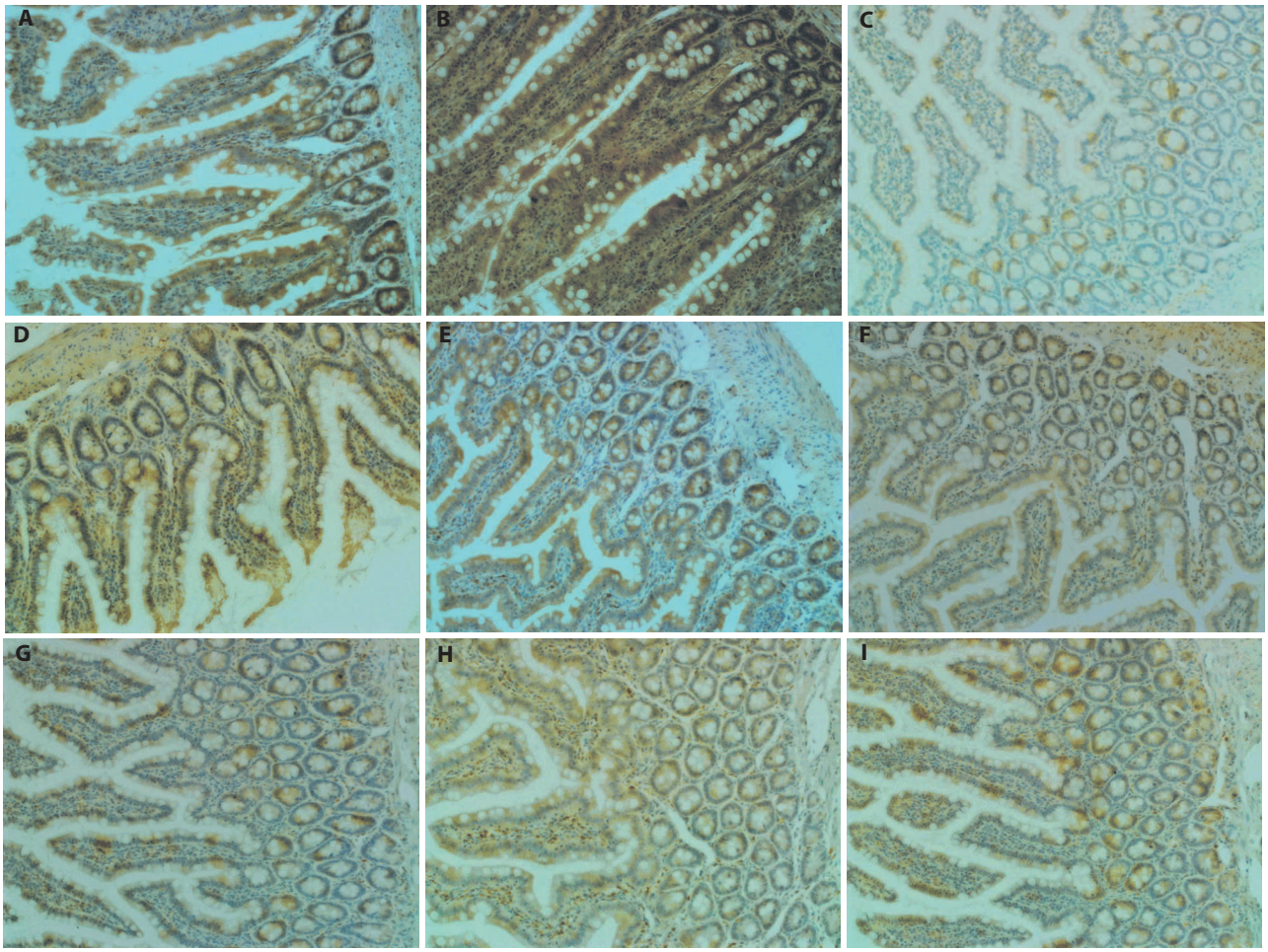

Figure 3 Expression of 5-HT in rat small intestine (Immunohistochemical staining, ×200)
A: stress-only group after 4 weeks of stress; B: stress-only group after 8 weeks of stress; C: naive control group; D: Sini San group after 4 weeks of stress; E: fluoxetine group after 4 weeks of stress; F: combination group after 4 weeks of stress; G: Sini San group after 8 weeks of stress; H: fluoxetine group after 8 weeks of stress; I: combination group after 8 weeks of stress.

group (P=0.026) and not significantly different from the control group (P>0.05). Fluoxetine-treated rats had higher serum 5-HT levels than rats in the Sini San or combination groups (P<0.05), but lower levels than the stress-only group (P=0.012). Within each group, the changes observed in serum 5-HT levels across the experiment were as follows: a consistent increase in the stress-only group (P=0.026); an increase in the Sini San group until 4 weeks of stress (P=0.034) followed by a reduction between 6 and 8 weeks of stress (P=0.026); and a peak in both the fluoxetine and combination groups after 2 weeks of stress (P=0.020) followed by a decrease after 4 weeks (P=0.034), and stabilizing between 6 to 8 weeks (P>0.05) (Table 4).

DISCUSSION

Modern medical research has demonstrated that the biological basis for depression is related to a lack of the monoamine neurotransmitter 5-HT, which plays a role in the regulation of emotion, memory, appetite, sexual function, and behavior in mammals.[7] The physiologi-

Table 4 Serum 5-HT concentrations

Group	Before the stress (n=40)	After 2 weeks of stress (n=40)	After 4 weeks of stress (n=40)	After 6 weeks of stress (n=20)	After 8 weeks of stress (n=20)
Naive control group	119±18	120±26[a]	125±20[a]	114±26[a]	120±25[a]
Stress-only group	126±27	178±36[b]	188±30[b]	209±23[b]	239±13[b]
Sini San group	120±21	161±30[b]	179±42[bc]	160±49[abc]	118±53[ac]
Fluoxetine group	121±20	168±24[b]	151±34[ab]	130±40[ab]	129±14[ab]
Combination group	123±23	160±34[abc]	145±36[ab]	120±39[ab]	117±10[ac]

Notes: naive rats were used as controls; the stress-only group were exposed to chronic mild stress (CMS) with no subsequent drug treatment; the Sini San group were exposed to CMS then received Sini San; the fluoxetine group were exposed to CMS then fluoxetine; the combination group were exposed to CMS then combined administration of Sini San and fluoxetine. [a]P<0.05, compared with the stress-only group; [b]P<0.05, compared with the control group; [c]P<0.05, compared with the fluoxetine group.

cal roles of 5-HT also include the stimulation of intestinal secretion and a direct action on smooth muscle, thus regulating gastrointestinal motility and secretion; conversely, physiological stimulation (eating) can promote the release of 5-HT.[8] In the present study, the persistent increase of 5-HT in the serum and small intestine in rats exposed to CMS suggests that chronic, unpredictable low-intensity stress can promote the release of 5-HT, resulting in a high sensitivity of the visceral afferent nerves and the enteric nervous system, which may activate a variety of neural active substances. This may in turn disrupt the chemical signals involved in the regulation of the brain-gut axis, resulting in abnormal gastrointestinal motility and visceral hypersensitivity, manifesting as diarrhea in rats. Changes in the levels of central and peripheral 5-HT may be useful biomarkers for the clinical diagnosis and treatment of depression. Modern medicine has proved that 5-HT mainly comes from the central nervous system and the intestinal tract. The central and peripheral metabolism of 5-HT occurs in two independent systems because it cannot cross the blood-brain barrier.[9] In the present study, the level of 5-HT in the hippocampus of rats in the stress-only group decreased as CMS continued, while that in serum and the small intestine steadily increased, suggesting that stress differentially affects central and peripheral 5-HT.

Depression is an affective disorder characterized by a persistent low mood. In Chinese medicine, depression falls into the category of Yu Zheng. The Traditional Chinese Medicine Sini San relieves the depressed liver and harmonizes the functions of liver and spleen. The unique law of compatibility, the therapeutic effects and the safety of Sini San have been confirmed by long-term clinical practice.[10] The results of the present study indicate that Sini San can be used in combination with fluoxetine to relieve symptoms of depression. In the later stages of treatment, fluoxetine can be decreased or stopped while Sini San is continued, to avoid the side-effects experienced after long-term use of the fluoxetine in the clinical treatment of depression.

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